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(71) Applicant *(for all designated States except US)*: UNIVERSITE LAVAL [CA/CA]; Cité Universitaire, Québec, Québec G1K 7P4 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: RAYMOND, Vincent [CA/CA]; Appartement 3, 858 Madeleine-de-Verchères, Québec, Québec G1S 4K5 (CA). MORISSETTE, Jean [CA/CA]; 3835 Jean-F. Grenon, Sainte-Foy, Québec G1S 3Y2 (CA). FALARDEAU, Pierre [CA/CA]; 1085 Marguerite-Bourgeoys, Sillery, Québec G1S 3Y2 (CA). COTE, Gilles [CA/CA]; 1395 De Godefroy, Sillery, Québec G1T 2E3 (CA). ANCTIL, Jean-Louis [CA/CA]; 1065 Marguerite-Bourgeoys, Sillery, Québec G1T 3Y1 (CA).

(74) Agents: DUBUC, Jean, H. et al.; Goudreau Gage Dubuc & Martineau Walker, The Stock Exchange Tower, Suite 3400, P.O. Box 242, Montreal, Québec H4Z 1E9 (CA).

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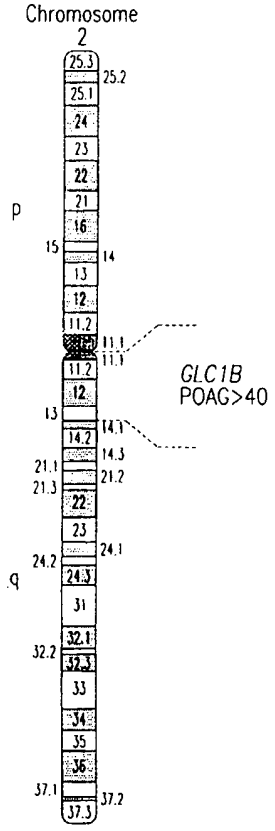
(54) Title: MOLECULAR DIAGNOSTIC OF GLAUCOMAS ASSOCIATED WITH CHROMOSOMES 2 AND 6

(57) Abstract

The present invention relates to haplotype analyses for the indirect detection of glaucoma. Based on the knowledge of linkage, two candidate genes for glaucoma one on chromosome 2, the other on chromosome 6, appear to be linked to a set of markers. The present invention provides the location of these markers and the primers for their amplification.



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TITLE OF THE INVENTION

MOLECULAR DIAGNOSTIC OF GLAUCOMAS ASSOCIATED WITH CHROMOSOMES 2 AND 6

FIELD OF THE INVENTION

The present invention relates to detecting glaucoma, especially in presymptomatic individuals using genetic linkage analyses such as haplotype analyses. The present invention also relates to the use of genetic recombinational events in the region spaning the haplotype markers to localise, identify and isolate the candidate gene.

BACKGROUND OF THE INVENTION

Glaucoma encompasses a complex of ocular-disease entities characterized by an optic neuropathy in which degeneration of retinal ganglion cells leads to a characteristic excavation of the head of the optic nerve (Shields et al., 1996, The Glaucomas, 2:717-725). Such damage causes progressive narrowing of the visual fields and, when uncontrolled, blindness. Affected people often have ocular hypertension defined as intraocular pressures consistently >21 mm Hg in both eyes. Although ocular hypertension is no longer an obligatory diagnostic criterion for glaucoma, it is still recognized as one of the most important risk factors (Wilson et al., 1996, The Glaucomas, 2:753-763). Until now, a diagnosis of glaucoma is made after observation of the characteristic atrophy of the optic nerve, which is associated with typical visual field defects.

In 1992, the World Health Organization estimated that, in the global population, 5.2 million people were blind as a result of glaucoma (Thylefors et al., 1994, World Health Organ. Bull., 72:323-326), making it the third leading cause of blindness worldwide. The most common form is adult-onset primary open-angle glaucoma (MIM 137760; McKusick, 1994, Johns Hopkins University Press, p. 272), which represents ~50% of all cases of glaucoma. Among Caucasians, this form of the disorder affects ~2% of the

population >45 years old (Leske, 1983, Am. Epidemiol., 118:166-191; Thylefors et al., 1994, supra; Wilson et al., 1996, supra). In African Americans, prevalence of adult-onset open-angle glaucoma is three to four times higher than that observed in White Americans. More than 15 million North Americans may have some form of glaucoma, but at least half of them may not be aware of it.

The glaucomas traditionally have been grouped into three categories: open angle, closed angle (also termed "angle closure"), and congenital. Each subtype has been further arbitrarily subdivided into *primary*, when the anterior chamber of the eye appears normal and no cause for glaucoma can be identified, or *secondary*, when glaucomas are caused by underlying ocular or systemic conditions (Shields et al., 1996, supra). Whereas the division between open and closed angles refers to the configuration of the irido-corneal angle in the anterior chamber of the eye, congenital glaucoma is used to define one of the many types of developmental glaucoma that usually occurs within the 1st year of life. The majority (60%-70%) of primary glaucomas are of the open-angle type. Primary open-angle glaucomas have been further subdivided into two groups according to age at onset, severity, and mode of inheritance: the more prevalent is middle- to late-age-onset chronic open-angle glaucoma (COAG), by convention diagnosed after age 35 years and characterized by its slow, insidious course (Shields et al., 1996, supra; Wilson et al., 1996, supra). The less common form, juvenile open-angle glaucoma (JOAG), occurs between 3 years of age and early adulthood and generally manifests highly elevated intraocular pressures with no angle abnormalities (Goldwyn et al., 1970, Arch. Ophtalmol., 84:579-582; François, 1980, Am. J. Ophtalmol., 3:429-449; Johnson et al., 1996*a*, The Glaucomas, 1:39-54).

Although the precise molecular defects leading to open-angle glaucomas remain partly unknown, numerous advances in basic and clinical sciences have begun to identify the molecular basis of glaucomas by mapping the gene loci involved in the disease process. Due to recent mapping successes, the different forms of glaucoma will be further identified by the

names of the loci to which they have been localized. According to the Human Genome Organization/Genome Database nomenclature, "*GLC*" is the general symbol for the glaucoma genes; "1", "2", and "3" are, respectively, the symbols for the open-angle, angle-closure, and congenital subtypes of glaucoma; and "A", "B", and "C" refer, respectively, to the first, second, or third gene mapped in each subgroup.

JOAG is a rare but aggressive form of glaucoma that usually segregates in an autosomal dominant fashion with high penetrance (Stokes, 1940, Arch. Ophthalmol., 24:885-909; Crombie et al., 1964, Br. J. Ophtalmol., 48:143-147; Lee et al., 1985, Ann. Ophtalmol., 17:739-741; Johnson et al., 1993, Ophthalmology, 100:524-529). In a single large American pedigree affected by an autosomal dominant form of JOAG, Sheffield et al. (1993, Nat. Genet., 4:47-50) located a gene responsible for this condition, at 1q21-q31. This locus, being the first open-angle glaucoma locus to be mapped, was named "*GLC1A*." The *GLC1A* disease gene consistently was associated with onset of the JOAG phenotype before the age of 70 years, highly elevated intraocular pressures, and typical excavation of the head of the optic nerve. Gonioscopy showed open angles with no anterior-chamber abnormalities. The *GLC1A* has subsequently been reported by Nguyen et.al in US Patent 5,606,043 to encode the trabecular meshwork induced glucocorticoid response (TIGR) gene. The first mutations within the TIGR gene that were related to glaucoma were first reported by Nguyen et al. (1997, Science, 275:668-670)

In August 1996, Stoilova et al. (1996, Genomics, 36:142-150) described the mapping of a second open-angle glaucoma locus, therefore named *GLC1B*, on chromosome 2cen-q13. To identify *GLC1B*, Sarfarazi's group selected 17 pedigrees ascertained either through the glaucoma registries of King's College Hospital in London or through the database of the International Glaucoma Association. Criteria for selection were bilateral open-angle glaucoma, autosomal dominant inheritance of the disease trait, at least two or three affected offspring and one living parent per family, and Caucasian origin

of the kindred. Six families containing a total of 16 affected individuals were linked to 2p11 -q 13 within an 11.2-cM region flanked by markers D2S2161 and D2S176 on chromosomal region 2cen-q13 (Stoilova et al., 1996, supra). The most interesting finding in these six *GLC1B* families was the high prevalence of glaucoma patients with normal intraocular pressures. Patients from these families demonstrated a clinical presentation with "low" to moderate intraocular pressures, onset in their late 40s, and a good response to medical therapy (Stoilova et al., 1996, supra). These authors concluded, appropriately, that the *GLC1B* region thus may encode a disease gene associated with cases of COAG that include those with moderately elevated intraocular pressures and those with normal-tension glaucoma. Eight other families who showed no linkage to this region provide evidence for genetic heterogeneity of the COAG phenotype.

The recent mapping of autosomal dominant iridogoniodysgenesis anomaly (IGDA) on 6p25, described by Mears et al. (1996, Am. J. Hum. Genet., 59:1321-1327), in the December issue of the *Journal*, adds one locus to this growing list of candidate regions. IGDA is characterized by iris hypoplasia, goniodysgenesis (abnormal iridocorneal angle), and juvenile glaucoma. In two families, one originating from the Maritime region of Canada and the second from south Wales, Mears et al. mapped the IGDA locus to an 8.3-cM region of 6p25, distal ro D6S477. Since IGDA is the result of aberrant migration or terminal induction of the neural crest cells, it is believed that the IGDA locus encodes a major gene involved in eye anterior-segment development (Mears et al., 1996, supra). Although these disorders showed some iridocorneal angle abnormalities, we should not be surprised if some families affected by open- or closed-angle glaucoma prove to be linked to one of these loci.

There thus remains a need to narrow down on the gene locus by following markers/candidate gene genetic linkage pattern.

The present invention seeks to meet these and other needs.

The present description refers to a number of documents, the content of which is herein incorporated by reference.

SUMMARY OF THE INVENTION

A large kindred, family PR-001, comprising more than 60 individuals spanning 3 living generations was identified with eight persons diagnosed with primary open-angle glaucoma (POAG). To identify the location of the disease-causing gene, genetic linkage studies were initiated exploiting a candidate/gene region approach. "Généthon" microsatellite markers were selected to cover 6 regions: *GLC1A, GLC1B, GLC1C,* 4q25, 6p25 and 11p13. It was discovered that glaucoma patients in family PR-001 harbored a common disease haplotype cosegregating with nine (9) Généthon markers located on 2cen-q13 at the *GLC1B* locus.

Another family with a very large pedigree was investigated having an initial diagnosis of autosomal dominant POAG, pedigree BV-001. This family comprised 192 individuals spanning 4 living generations. To identify the location of the disease-causing gene, a genetic linkage study exploiting a candidate/gene region approach. "Généthon" microsatellite markers were also selected to cover 6 regions: *GLC1A, GLC1B, GLC1C,* 4q25, 6p25 and 11p13. Eighty-three persons were assessed. Twenty-one patients were diagnosed with autosomal dominant glaucoma. Nine other persons showed ocular hypertension (OHT). Out of those 30 patients, 9 also displayed iris hypoplasia and/or some excess tissue in the iridocorneal angle. For linkage analysis, OHT and glaucoma patients were considered affected. Tight linkage was observed between the affected phenotype and 7 markers on 6p25; a maximum lod score of 8.81 obtained with UT7184 (D6S967). The same characteristic haplotype was recognized in all patients. Key affected recombinants confined the disease region within a 5 cM interval between loci D6S344 and D6S1713. Our data demonstrated that the *6p25* disease gene can cause wide variable expressivity, ranging from developmental anomalies in the anterior chamber of the eye to

glaucoma that may be diagnosed as POAG. These findings further suggested that this disease gene and the iridogoniodysgenesis gene also mapped to the same region may be allelic.

Accordingly, the present invention seeks to provide a method for identifying individuals that are at risk for inheriting glaucoma whether these individuals are genotypically normal, symptomatic or presymptomaic for glaucomas. The method described herein relates to haplotype analyses based on 9 and 10 contiguous markers on chromosomes 2 and 6 respectively. The genetic markers provided for in this invention span the microsatellite regions of chromosome 2 cen-q13 and chromosome 6p ter-6p 25 and are 11.4 cM and 4 cM in length, respectively. These markers are polymorphic and are therefore informative of the pattern of heritability of the particular candidate gene region. A characterised haplotype profile as defined by the markers encompassing the specific target region on a chromosome in a specific pedigree or a population, are found to be in association with a particular gene locus that can be followed and traced from generation to generation. The heritability of these candidate genes causing disease can be predicted indirectly by their linkage or association with a haplotype profile.

The present invention further relates to identifying the morbid chromosome region, the region of the chromosome comprising a mutated gene. By analysing the haplotype in members of a well defined family the applicant is using this information to close in on the gene locus. By following naturally occuring events such as genetic recombinational events that can be detected by the inheritance of non-parental haplotype pattern with or without an association with a disease causing gene.

Accordingly, the present invention seeks to provide a method for identifying individuals that are at risk for inheriting glaucoma whether these individuals are genotypically normal, symptomatic or presymptomaic for glaucomas. The method described herein relates to haplotype analyses based on 9 and 10 contiguous markers on chromosomes 2 and 6 respectively. The

genetic markers provided for in this invention span the microsatellite regions of chromosome 2 cen-q13 and chromosome 6p ter-6p 25. These markers are polymorphic and are therefore informative of the pattern of heritability of the particular chromosomal region. A characterised haplotype profile as defined by the markers encompassing the specific target region on a chromosome in a specific pedigree or a population, are found to be in linkage or association with a particular gene locus that can be followed and traced from generation to generation. The heritability of these genes can be predicted indirectly by their linkage or association with a haplotype profile.

In a particular embodiment of this invention it is provided that if the gene locus is defective or mutant such that the defect or mutation gives rise to a non- normal state in an individual, the analyses of a haplotype profile provides the means for indirectly predicting the heritability of the said defect or mutant.

In another embodiment of the present invention, there is provided a kit comprising all the necessary reagents to carry out the herein described methods of detection. As known to the person of ordinary skill, the kit comprises container means comprising oligonucleotide sequences or antibodies (or binding proteins) and reagents such as washing reagents, reagents for detection purposes and the like. It will also be readily recognized that the nucleic acid sequences or antibodies of the present invention could be incorporated into established kit formats.

The invention in addition provides the means to identify by haplotype analyses a method for following a mutant allele in a pedigree or a population and establishing a linkage or an association with the said haplotype and as yet unknown disease causing gene of glaucoma that are caused by defects in the gene loci on chromosome 2 and/ or 6 in an as yet unknown gene/s relating to glaucoma.

In a more particular embodiment the invention provides the suitable means for tracing the heritability pattern for the candidate genes *GLC1B*

and *GLC6p25* that are associated and linked to defined haplotype profile on chromosome 2 and 6 respectively.

A further advantage of this invention is the identification of presymptomatic individuals using the herein described methods and medically intervening in the disease process. Thus, obviating the impact of inheriting a mutant allele causing disease, by medically disrupting the initiation or progression of the disease.

The present invention also seeks to provide well defined contiguous regions on chromosomes 2 and 6 that comprise the candidate genes *GLC1B* and *GLC6p25* respectively, wherein mutations in the said genes give rise to the development of glaucoma. The identification of these markers spanning the area of the chromosome to which the gene loci are associated, are in the microsatellite regions of the chromosomes and are contiguous. These markers define a particular haplotype profile that is linked to a gene locus, this knowledge provides the means in combination with other naturally occurring genetic events such as, but not limited to genetic recombination, to narrow the search and potentiate the means to localise, identify and isolate the candidate gene.

In accordance with the present invention there is provided the pairs of amplification primers for each of the markers derived from the microsatellite regions of chromosomes 2 and 6. This invention is not limited to the primers disclosed, since to a person skilled in the art, it would be easy to design other primers to serve the purpose of this invention. Any pair of primers amplifying across the polymorphic region can be used. These amplification primers can be of any length and hybridize to sequences flanking the nucleotide region comprising the polymorphism, such that amplification product of the polymorphic sequence is detectable.

The invention extends to any products useful for the carrying out of this analyses such as DNA primers, kits and such.

The present invention provides a set of markers forming haplotypes which can be used for the detection, assessment or prognosis of glaucoma.

DEFINITIONS AND TECHNOLOGICAL BACKGROUND

Nucleotide sequences are presented herein by single strand, in the 5' to 3' direction, from left to right, using the one letter nucleotide symbols as commonly used in the art and in accordance with the recommendations of the IUPAC-IUB Biochemical Nomenclature Commission.

The present description refers to a number of routinely used recombinant DNA (rDNA) technology terms. Nevertheless, definitions of selected examples of such rDNA terms are provided for clarity and consistency.

As used herein, "isolated nucleic acid molecule", refers to a polymer of nucleotides. Non-limiting examples thereof include DNA and RNA molecules purified from their natural environment.

The term "DNA segment", is used herein, to refer to a DNA molecule comprising a linear stretch or sequence of nucleotides. This sequence when read in accordance with the genetic code, can encode a linear stretch or sequence of amino acids which can be referred to as a polypeptide, protein, protein fragment and the like.

The terminology "amplification pair" or "primer pair" refers herein to a pair of oligonucleotides (oligos) of the present invention, which are selected to be used together in amplifying a selected nucleic acid sequence by one of a number of types of amplification processes, preferably a polymerase chain reaction. Other types of amplification processes include ligase chain reaction, strand displacement amplification, or nucleic acid sequence-based amplification, as explained in greater detail below. As commonly known in the art, the oligos are designed to bind to a complementary sequence under selected conditions.

The nucleic acid (i.e. DNA or RNA) for practicing the present invention may be obtained according to well known methods.

Oligonucleotide probes or primers of the present invention may be of any suitable length, depending on the particular assay format and the particular needs and targeted genomes employed. In general, the oligonucleotide probes or primers are at least 12 nucleotides in length, preferably between 15 and 24 nucleotides, and they may be adapted to be especially suited to a chosen nucleic acid amplification system. As commonly known in the art, the oligonucleotide probes and primers can be designed by taking into consideration the melting point of hydrizidation thereof with its targeted sequence (Sambrook et al., 1989, Molecular Cloning - A Laboratory Manual, 2nd Edition, CSH Laboratories; Ausubel et al., 1989, in Current Protocols in Molecular Biology, John Wiley & Sons Inc., N.Y.).

"Nucleic acid hybridization" refers generally to the hybridization of two single-stranded nucleic acid molecules having complementary base sequences, which under appropriate conditions will form a thermodynamically favored double-stranded structure. Examples of hybridization conditions can be found in the two laboratory manuals referred above (Sambrook et al., 1989, *supra* and Ausubel et al., 1989 supra) and are commonly known in the art. In the case of a hybridization to a nitrocellulose filter, as for example in the well known Southern blotting procedure, a nitrocellulose filter can be incubated overnight at 65°C with a labeled probe in a solution containing 50% formamide, high salt (5 x SSC or 5 x SSPE), 5 x Denhardt's solution, 1% SDS, and 100 µg/ml denatured carried DNA (i.e. salmon sperm DNA). The non-specifically binding probe can then be washed off the filter by several washes in 0.2 x SSC/0.1% SDS at a temperature which is selected in view of the desired stringency: room temperature (low stringency), 42°C (moderate stringency) or 65°C (high stringency). The selected temperature is based on the melting temperature (Tm) of the DNA hybrid. Of course, RNA-DNA hybrids can also be formed and detected. In such cases, the

conditions of hybridization and washing can be adapted according to well known methods by the person of ordinary skill. High stringency conditions will be preferably used (Sambrook et al.,1989, supra).

Probes of the invention can be utilized with naturally occurring sugar-phosphate backbones as well as modified backbones including phosphorothioates, dithionates, alkyl phosphonates and α-nucleotides and the like. Modified sugar-phosphate backbones are generally taught by Miller, 1988, Ann. Reports Med. Chem. 23:295 and Moran et al., 1987, Nucleic acid molecule. Acids Res., 14:5019. Probes of the invention can be constructed of either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), and preferably of DNA.

The types of detection methods in which probes can be used include Southern blots (DNA detection), dot or slot blots (DNA, RNA), and Northern blots (RNA detection). Although less preferred, labelled proteins could also be used to detect a particular nucleic acid sequence to which it binds. Other detection methods include kits containing probes on a dipstick setup and the like.

Although the present invention is not specifically dependent on the use of a label for the detection of a particular nucleic acid sequence, such a label might be beneficial, by increasing the sensitivity of the detection. Furthermore, it enables automation. Probes can be labelled according to numerous well known methods (Sambrook et al., 1989, supra). Non-limiting examples of labels include ^{3}H, ^{14}C, ^{32}P, and ^{35}S. Non-limiting examples of detectable markers include ligands, fluorophores, chemiluminescent agents, enzymes, and antibodies. Other detectable markers for use with probes, which can enable an increase in sensitivity of the method of the invention, include biotin and radionucleotides. It will become evident to the person of ordinary skill that the choice of a particular label dictates the manner in which it is bound to the probe.

As commonly known, radioactive nucleotides can be incorporated into probes of the invention by several methods. Non-limiting

examples thereof include kinasing the 5' ends of the probes using gamma ^{32}P ATP and polynucleotide kinase, using the Klenow fragment of Pol I of *E. coli* in the presence of radioactive dNTP (i.e. uniformly labelled DNA probe using random oligonucleotide primers in low-melt gels), using the SP6/T7 system to transcribe a DNA segment in the presence of one or more radioactive NTP, and the like.

As used herein, "oligonucleotides" or "oligos" define a molecule having two or more nucleotides (ribo or deoxyribonucleotides). The size of the oligo will be dictated by the particular situation and ultimately by the particular use thereof, and adapted accordingly by the person of ordinary skill. An oligonucleotide can be synthetised chemically or derived by cloning according to well known methods.

As used herein, a "primer" defines an oligonucleotide which is capable of annealing to a target sequence, thereby creating a double stranded region which can serve as an initiation point for DNA synthesis under suitable conditions.

Amplification of a selected, or target, nucleic acid sequence may be carried out by a number of suitable methods. See generally Kwoh et al., 1990, (Am. Biotechnol. Lab. 8:14-25). Numerous amplification techniques have been described and can be readily adapted to suit the particular needs of a person of ordinary skill. Non-limiting examples of amplification techniques include polymerase chain reaction (PCR), ligase chain reaction (LCR), strand displacement amplification (SDA), transcription-based amplification, the Qβ replicase system and NASBA (Kwoh et al., 1989, Proc. Natl. Acad. Sci. USA 86, 1173-1177; Lizardi et al., 1988, BioTechnology 6:1197-1202; Malek et al., 1994, Methods Mol. Biol., 28:253-260; and Sambrook et al., 1989, *supra*). Preferably, amplification will be carried out using PCR.

Polymerase chain reaction (PCR) is carried out in accordance with known techniques. See, e.g., U.S. Pat. Nos. 4,683,195; 4,683,202; 4,800,159; and 4,965,188 (the disclosures of all three U.S. Patent are

incorporated herein by reference). In general, PCR involves, a treatment of a nucleic acid sample (e.g., in the presence of a heat stable DNA polymerase) under hybridizing conditions, with one oligonucleotide primer for each strand of the specific sequence to be detected. An extension product of each primer which is synthesized is complementary to each of the two nucleic acid strands, with the primers sufficiently complementary to each strand of the specific sequence to hybridize therewith. The extension product synthesized from each primer can also serve as a template for further synthesis of extension products using the same primers. Following a sufficient number of rounds of synthesis of extension products, the sample is analysed to assess whether the sequence or sequences to be detected are present. Detection of the amplified sequence may be carried out by visualization following EtBr staining of the DNA following gel electrophoresis, or using a detectable label in accordance with known techniques, and the like. For a review on PCR techniques (see PCR Protocols, A Guide to Methods and Amplifications, Michael et al., Eds, Acad. Press, 1990).

Ligase chain reaction (LCR) is carried out in accordance with known techniques (Weiss, 1991, Science 254:1292). Adaptation of the protocol to meet the desired needs can be carried out by a person of ordinary skill. Strand displacement amplification (SDA) is also carried out in accordance with known techniques or adaptations thereof to meet the particular needs (Walker et al., 1992, Proc. Natl. Acad. Sci. USA 89:392-396; and ibid., 1992, Nucleic Acids Res. 20:1691-1696).

As used herein, the term "gene" is well known in the art and relates to a nucleic acid sequence defining a single protein or polypeptide. A "structural gene" defines a DNA sequence which is transcribed into RNA and translated into a protein having a specific amino acid sequence thereby giving rise the a specific polypeptide or protein. It will be readily recognized by the person of ordinary skill, that the nucleic acid sequences of the present invention can be incorporated into anyone of numerous established kit formats which are well known in the art.

The term "expression" defines the process by which a structural gene is transcribed into mRNA (transcription), the mRNA is then being translated (translation) into one polypeptide (or protein) or more.

The terminology "expression vector" defines a vector or vehicle, as described above, but designed to enable the expression of an inserted sequence following transformation into a host. The cloned gene (inserted sequence) is usually placed under the control of control element sequences such as promoter sequences. The placing of a cloned gene under such control sequences is often referred to as being "operably linked" to control elements or sequences.

Expression control sequences will vary depending on whether the vector is designed to express the operably linked gene in a prokaryotic or eukaryotic host or both (shuttle vectors) and can additionally contain transcriptional elements such as enhancer elements, termination sequences, tissue-specificity elements, and/or translational initiation and termination sites.

As used herein, the designation "functional derivative" denotes, in the context of a functional derivative of a sequence, whether nucleic acid or amino acid sequence, a molecule that retains a biological activity (either functional or structural) that is substantially similar to that of the original sequence. This functional derivative or equivalent may be a natural derivative or may be prepared synthetically. Such derivatives include amino acid sequences having substitutions, deletions, or additions of one or more amino acids, provided that the biological activity of the protein is conserved. The same applies to derivatives of nucleic acid sequences which can have substitutions, deletions, or additions of one or more nucleotides, provided that the biological activity of the sequence is generally maintained. When relating to a protein sequence, the substituting amino acid has chemico-physical properties which are similar to that of the substituted amino acid. The similar chemico-physical properties include, similarities in charge, bulkiness, hydrophobicity, hydrophylicity and the like. The term "functional derivatives" is intended to

include "fragments", "segments", "variants", "analogs" or "chemical derivatives" of the subject matter of the present invention.

Thus, the term "variant" refers herein to a protein or nucleic acid molecule which is substantially similar in structure and biological activity to the protein or nucleic acid of the present invention.

The functional derivatives of the present invention can be synthesized chemically or produced through recombinant DNA technology. All these methods are well known in the art.

As used herein, "chemical derivatives" is meant to cover additional chemical moieties not normally part of the subject matter of the invention. Such moieties could affect the physico-chemical characteristic of the derivative (i.e. solubility, absorption, half life and the like, decrease of toxicity). Such moieties are exemplified in Remington's Pharmaceutical Sciences (1980). Methods of coupling these chemical-physical moieties to a polypeptide are well known in the art.

The term "allele" defines an alternative form of a gene or DNA sequence at a specific chromosomal location (locus). At each autosomal locus an individual possesses two alleles, one inherited from the father and one from the mother.

As commonly known, a "mutation" is a detectable change in the genetic material which can be transmitted to a daughter cell. As well known, a mutation can be, for example, a detectable change in one or more deoxyribonucleotide. For example, nucleotides can be added, deleted, substituted for, inverted, or transposed to a new position. Spontaneous mutations and experimentally induced mutations exist. The result of a mutations of nucleic acid molecule is a mutant nucleic acid molecule. A mutant polypeptide can be encoded from this mutant nucleic acid molecule.

As used herein, the term "purified" refers to a molecule having been separated from a cellular component. Thus, for example, a "purified

protein" has been purified to a level not found in nature. A "substantially pure" molecule is a molecule that is lacking in all other cellular components.

As used herein, the terminology "target region" defines a DNA region for which preliminary sequence data is sufficiently available to enable the design of a first primer pair which will, under appropriate conditions, give rise to a recognizable extension product. The target region is determined and defined by the available sequence data available for the particular genome analysed, and by the limits in the amplification method used. For PCR, for example, the conditions permit extension products to reach about 2000 nucleotides. The target region should thus be between about 50 to about 2000 nucleotides. Preferably between about 200 and about 1000. Since sequence information can be clustered, some genes might have several target regions. In any event, the mutagenesis conditions should be adapted so as to enable an insertional mutagenesis of all targeted regions. In essence, a person of ordinary skill will adapt the mutagenesis scheme so as to permit saturation mutagenesis of the DNA to be analysed.

A Restriction Endonuclease (also restriction enzyme) is an enzyme that has the capacity to recognize a specific base sequence (usually 4, 5, or 6 base pairs in length) in a DNA molecule, and to cleave the DNA molecule at every place where this sequence appears. For example, *Eco*RI recognizes the base sequence GAATTC/CTTAAG.

Restriction Fragment. The DNA molecules produced by digestion with a restriction endonuclease are referred to as restriction fragments. Any given genome can be digested by a particular restriction endonuclease into a discrete set of restriction fragments.

Agarose Gel Electrophoresis. To detect a polymorphism in the length of restriction fragments, an analytical method for fractionating double-stranded DNA molecules on the basis of size is required. The most commonly used technique (though not the only one) for achieving such a fractionation is agarose gel electrophoresis. The principle of this method is that

DNA molecules migrate through the gel as though it were a sieve that retards the movement of the largest molecules to the greatest extent and the movement of the smallest molecules to the least extent. Note that the smaller the DNA fragment, the greater the mobility under electrophoresis in the agarose gel. This method can be used to detect amplified DNA fragments without undergoing any other manipulation.

The DNA fragments fractionated by agarose gel electrophoresis can be visualized directly by a staining procedure if the number of fragments included in the pattern is small. The DNA fragments of genomes can be visualized successfully. However, most genomes, including the human genome, contain far too many DNA sequences to produce a simple pattern of restriction fragments. For example, the human genome is digested into approximately 1,000,000 different DNA fragments by *Eco*RI. In order to visualize a small subset of these fragments, a methodology referred to as the Southern hybridization procedure can be applied.

"Single Strand Conformational Polymorphism (SSCP)" refers to a method for detecting the presence of a base pair change in an amplified DNA fragment. The method involves denaturing the double stranded amplified DNA and comparing the band pattern in a known non-mutant fragment to that of an unknown fragment. A shift in the band pattern is indicative of a base pair change.

The designation "gene therapy" defines an attempt to treat disease by genetic modification of the cells of a patient.

"Artificially Created Restriction Site (ACRS)" refers to a method for detection a known base change in a DNA sequence. It involves the designing of a primer that may either create or obviate a restriction site in the vicinity of known base change, such that the restriction endonuclease used can have a different digestion pattern for the changed and unchanged base.

The invention also relates to polypeptides encoded by the nucleic acid sequences of the present invention. Such antibodies can be raised from such polypeptides.

In general, techniques for preparing antibodies (including monoclonal antibodies and hybridomas) and for detecting antigens using antibodies are well known in the art (Campbell, 1984, In "Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology", Elsevier Science Publisher, Amsterdam, The Netherlands) and in Harlow et al., 1988 (in: Antibody - A Laboratory Manual, CSH Laboratories). The present invention also provides polyclonal, monoclonal antibodies, or humanized versions thereof, chimeric antibodies and the like which inhibit or neutralize their respective interaction domains and/or are specific thereto.

The present invention also provides antisense nucleic acid molecules which can be used for example to decrease or abrogate the expression of the normal allele. An antisense nucleic acid molecule according to the present invention refers to a molecule capable of forming a stable duplex or triplex with a portion of its targeted nucleic acid sequence (DNA or RNA). The use of antisense nucleic acid molecules and the design and modification of such molecules is well known in the art as described for example in WO 96/32966, WO 96/11266, WO 94/15646, WO 93/08845, and USP 5,593,974. Antisense nucleic acid molecules according to the present invention can be derived from the nucleic acid sequences and modified in accordance to well known methods. For example, some antisense molecules can be designed to be more resistant to degradation to increase their affinity to their targeted sequence, to affect their transport to chosen cell types or cell compartments, and/or to enhance their lipid solubility by using nucleotide analogs and/or substituting chosen chemical fragments thereof, as commonly known in the art.

From the specification and appended claims, the term therapeutic agent should be taken in a broad sense so as to also include a combination of at least two such therapeutic agents. Further, the DNA segments

or proteins according to the present invention can be introduced into individuals in a number of ways. For example, erythropoietic cells can be isolated from the afflicted individual, transformed with a DNA construct according to the invention and reintroduced to the afflicted individual in a number of ways, including intravenous injection. Alternatively, the DNA construct can be administered directly to the afflicted individual, for example, by injection in the bone marrow. The DNA construct can also be delivered through a vehicle such as a liposome, which can be designed to be targeted to a specific cell type, and engineered to be administered through different routes.

For administration to humans, the prescribing medical professional will ultimately determine the appropriate form and dosage for a given patient, and this can be expected to vary according to the chosen therapeutic regimen (i.e DNA construct, protein, cells), the response and condition of the patient as well as the severity of the disease.

Composition within the scope of the present invention should contain the active agent (i.e. fusion protein, nucleic acid, and molecule) in an amount effective to achieve the desired therapeutic effect while avoiding adverse side effects. Non-limiting examples of active agents include the mutant protein according to the present invention a nucleic acid molecule encoding such a mutant protein, an antisense molecule to a normal allele according to the present invention, etc. Typically, the nucleic acids in accordance with the present invention can be administered to mammals (i.e. humans) in doses ranging from 0.005 to 1 mg per kg of body weight per day of the mammal which is treated. Pharmaceutically acceptable preparations and salts of the active agent are within the scope of the present invention and are well known in the art (Remington's Pharmaceutical Science, 16th Ed., Mack Ed.). For the administration of polypeptides, antagonists, agonists and the like, the amount administered should be chosen so as to avoid adverse side effects. The dosage will be adapted by the clinician in accordance with conventional factors such as

the extent of the disease and different parameters from the patient. Typically, 0.001 to 50 mg/kg/day will be administered to the mammal.

The term "Genetic recombination" refers to the physical exchange of parts between two homologous sequences and resulates in an offspring with a different genotype from either parental genotype.

The term "Microsatellite DNA" defines a small array (often less than 0.1 kb) of tandem repeats of a very simple sequence, often between 1 and 4 bp.

The term "Pedigree" refers to a diagram that shows ancestral relationships among members of a family over several generations.

The term "Haplotype" denotes a series of alleles found at linked loci on a single (paternal or maternal) chromosome.

The term "Population" refers to a collection of individuals sharing at least one common characteristic.

The term "Mendelian population" defines a group of sexually interbreeding or potentially interbreeding individuals in a defined area.

The term "Marker" defines a genetic marker which is a polymorphic DNA or protein sequence deriving from a single chromosomal location, which is used in genetic mapping.

The term "Morgan" refers to a unit of genetic distance corresponding to a length of DNA which, on average, undergoes one crossover per individual chromatid strand. On average, there are about 52 chiasmata in human male meiosis and so the total male genetic map distance is 26 Morgans or 2600 cM.

The term "CentiMorgan (cM)" denotes a unit of genetic distance equivalent to a 1% probability of recombination during meiosis (see Morgan). One CentiMorgan is equivalent, on average, to a physical distance of approximately 1 megabase in the human genome. One CentiMorgan is 100 of a Morgan = 0.01 M.

The term "Linkage" refers to the tendency of genes or other DNA sequences at specific loci to be inherited together as a consequence of their physical proximity on a single chromosome.

The term "linkage disequilibirum" (also called allelic association) defines a nonrandom association of alleles at linked loci.

The term "Lod score" refers to a measure of the likelihood of genetic linkage between loci. A lod score greater that +3 is often taken as evidence of linkage; one that is less than -2 is often taken as evidence against linkage.

BRIEF DESCRIPTION OF THE DRAWINGS

Having thus generally described the invention, reference will now be made to the accompanying drawings, showing by way of illustration a preferred embodiment thereof, and in which:

Fig. 1 shows the genetic map of chromosome 2 with the locus *GLC1B* on the long arm of chromosome 2 region 2 cen-2q 13;

Fig. 2 shows the phenotypic status and segregation analyses of the *GLC1B* disease haplotype in family PR. All living individuals were investigated for glaucoma and genotyped with microsatellite markers spanning the *GLC1B* locus. Selected AFM markers with their corresponding GDB number are represented and the sizes of the alleles associated with the *GLC1B* disease haplotype observed for each marker in pedigree PR are boxed in black. Glaucoma patients are depicted by solid balck symbols, unaffected individuals by open symbols, and deceased subjects reported as blind by at least two independent family members by a black quadrant in the upper left corner of their respective symbols. OHT persons are represented by open symbols containing a central solid dot. A solid black box indicates the common *GLC1B* disease haplotype. The right side of each phased haplotype indicates the haplotype inherited from the father; the left side indicates the haplotype inherited from the mother.

Fig. 3 shows the genetic map of chromosome 6 with the locus *GLC6p25* on the short arm of chromosome 6 region 6p 25-6p ter

Fig. 4 shows the phenotypic status and segregation analyses of the *GLC6p25* disease haplotype in family BV. All living individuals were investigated for glaucoma and genotyped with microsatellite markers spanning the *GLC6p25* locus. Selected AFM (Généthon) and UTAH markers with their corresponding GDB number are represented and the sizes of the alleles associated with the *GLC6p25* disease haplotype observed for each marker in pedigree BV are boxed in black. Glaucoma patients are depicted by solid black symbols, unaffected individuals by open symbols, and deceased subjects reported as blind by at least two independent family members by a black quadrant in the upper left corner of their respective symbols. OHT persons are represented by open symbols containing a central solid dot. A solid black box indicates the common *GLC6p25* disease haplotype. The right side of each phased haplotype indicates the haplotype inherited from the father; the left side indicates the haplotype inherited from the mother. Present ages of normal and OHT patients as well as ages of affected carriers at time of diagnosis are depicted above their respective symbols.

Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of preferred embodiments with reference to the accompanying drawing which is exemplary and should not be interpreted as limiting the scope of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

At its broadest, the invention comprises the detection of the presence of at least two genes for glaucoma by analyzing human chromosomes 2 and 6 for DNA polymorphism wherein the gene loci for glaucoma are linked. The glaucoma gene on chromosome 2 is *GLC1B* and on chromosome 6 is *GLC6p25*.

Genetic maps of the human genome can be exploited to rapidly locate human monogenic disorders. The final version of the Généthon linkage map, which spans close to 100 % of the human genome, was published in March 1996 (Dib et al., 1996, Nature, 380:152-154). This map consists of 5,264 short tandem (AC/TG)n repeat polymorphisms with a mean heterozygosity of 70%.

The nomenclature system for the markers is well known in the field. The nomenclature used is decided by the Human Genome Organization (HUGO) nomenclature committee. It is as follows: for anonymous DNA sequences, the convention is to use D which is equivalent to DNA followed by 1-22, X or Y to denote the chromosomeal number and location, then S stands for a unique segment and finally a serial number. For example, marker D2S2161 is a DNA marker located on chromosome 2 representing a unique segment. Its serial number is 2161.

The nomenclature for the glaucoma genes is the following: "*GLC*" is the general symbol for the glaucoma genes; "1", "2" and "3" are, respectively, the symbols for the open-angle, angle-closure, and congenital subtypes of glaucoma; and, "A", "B" and "C" refer, respectively, to the first, second, or third gene mapped in each subgroup. For example, the *GLC1A* locus was the first open-angle glaucoma locus to be mapped, in this case to chromosome 1q23-q25 in 1993. It was later identified as the trabecular meshwork inducible glucocorticoid response gene product (*TIGR*) (Stone et al., 1997, Science, 275: 668-670).

These markers are accessible to all individuals. The central data resource for the human gene mapping effort is the Genome Data Base (GDB). It was established at Johns Hopkins University, School of Medicine. GDB is updated regularly. It collects, organizes, stores and distributes human genome mapping information. GDB is accessible eletronically at WWW-URL: http://gdbwww.gdb.org/.

Alternatively, all the markers disclosed herein, except D6S967, are short (CA)n repeat markers that have been developed in the Généthon laboratory near Paris, France. These markers are also accessible eletronically at WWW-URL: http://www.genethon.fr/.

Therefore markers are accessible either at GDB or at Généthon.

The use of amplification primers flanking sequences in the microsatellite region linked to these genes result in amplified sequences that vary in length. The variations are due to nucleotide repeats in the microsatellite region that are polymorphic and these polymorphic sequences are used as markers. A set of these associated polymorphic markers form a haplotype profile in an individual. As well, a defined haplotype in an individual can be used to trace the inheritance pattern and in this way can be used for indirectly identifying the presence of the glaucoma gene. An allele comprising the mutant gene is called a morbid allele. Any method of analysis which yields the linkage results of a polymorphism with the mutant gene *GLC1B* or *GLC6p25* on either chromosome 2 or 6 respectively can be utilized.

A set of markers defining the haplotype may be inherited together and the haplotype may remain identical when compared to the parental haplotype, or the region comprising the haplotype can undergo genetic recombination to result in haplotypes that are different from the parental haplotypes. Using naturally occuring recombinational events followed by parental and progeny haplotype analyses one can include or exclude a gene locus by following the presence of the mutant gene on a particular non-recombinant or recombinant haplotype. In this way one can narrow the position of the gene locus and use this information to localise identify and isolate the candidate gene.

The present invention is illustrated in further detail by the following non-limiting examples.

EXAMPLE 1

1.1 Pedigrees and ophthalmologic assessments

1.1.1 Pedigree reconstitutions

The pedigree genealogies were reconstituted using the registers compiled from the Catholic parish records, which systematically list births, marriages, and deaths of 98% of the Québec population.Validation of the family tree and new data on recent births were obtained through interviews with key family members. The Archives Nationales du Québec, the Québec Civil register, and the Institut de recherche sur l'étude des populations (IREP) data base (Bouchard and De Braekeleer 1991) were also consulted.

1.1.2 Ophthalmologic investigations

All subjects, affected or not, gave informed consent before entering the study. As described in Morissette et al. (1995, Am. J. Hum. Genet., 56:1431-1442), clinical assessments comprised complete ophthalmologic evaluation, including best corrected visual acuity; optic disk examination; slit-lamp biomicroscopy; applanation tonometry; gonioscopy; and visual-field evaluation. Three criteria were required for primary open-angle glaucoma (POAG) diagnosis: a) intraocular pressures above 22 mm Hg in both eyes, b) characteristic optic disk damage and/or visual field impairment, and c) grade III or IV (open-angle) gonioscopy. In the absence of optic disk damage or visual-field alteration, subjects with intraocular pressures above 22 mm Hg in both eyes and grade III or IV gonioscopy were diagnosed with ocular hypertension (OHT). Members of the families were considered normal when they presented normal optic disks and showed highest intraocular pressures ever recorded at 22 mm Hg or less. Persons with other forms of glaucomas, including grade 0 (closed angle); grade I or II (narrow-angle); congenital; and secondary glaucomas, or with other nonglaucomatous ocular disorders were considered unaffected. Blindness in deceased ancestors was confirmed by at least two independent sources. Iridogoniodysgenesis, iris hypoplasia and abnormal tissue in the angle was carefully assessed.

EXAMPLE 2

2.1 Source of DNA

Blood samples were obtained from direct descendants of the founder in each family as well as spouses of affected patients with children; from each, 20 ml of blood was drawn by venipuncture in heparinized tubes. One additional 10 ml blood sample was drawn from selected key subjects to establish lymphoblastoid cell lines using the method of Anderson et al. (1984, In Vitro, 20:856-858).

2.2 Isolation of DNA

DNA was extracted from whole blood using the guanidine hydrochloride-proteinase K method developed by Jeanpierre (1987, Nucl. Acids. Res. 15:9611-9611).

2.3 Genotyping procedures

To accelerate genotyping, we used a protocol similar to the procedure of Vignal et al. (1993, Methods in molecular genetics, Academic Press, 1:211-221) which was derived from the multiplex sequencing technique of Church and Kieffer-Higgins (1988, Science 240:185-188). Briefly, polymerase chain reactions (PCR) were performed in a total volume of 50 ml containing 100 ng of genomic DNA, 50 pmol of each primer, 125 mM dNTPs, 50 mM KCl, 10 mM Tris (pH 9), 1.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton X-100, and 1 U Taq polymerase (Perkin-Elmer-Cetus). Amplifications were carried out using a "hot-start" procedure. Taq polymerase was added after a 5-min denaturation step at 96°C. Samples were then processed through 35 cycles of denaturation (94°C for 40 s) and annealing (55°C for 30 s), followed by one last step of elongation (2 min at 72°C). Usually, three amplification products synthesized with separate primer sets on identical DNA samples were coprecipitated and comigrated in a single lane of 6% polyacrylamide denaturing gels. Separated products were then transferred onto Hybond N^+ nylon membranes (Amersham), hybridized with a $(CA)_{20}$ oligomer 3' labeled with Digoxigenin-11-ddUTP, and detected by chemiluminescence using the DIG system (Boehringer-Mannheim) with Kodak XAR-5 films. Genotypes were scored relative to reference alleles of

the mother of the CEPH family 1347 (individual 134702). Genotyping was repeated upon detection of incompatibilities or recombination events.

2.4 Selection of primers

AFM markers have been defined previously (Gyapay et al., 1994, Nature Genet., 7:246-339; Dib et al., 1996, supra). One Utah marker (D6S967) was selected from GDB. DNA primer sequences were the following:

CHROMOSOME 2

MARKER NAME	SEQ. ID. NO.	STRAND PRIMER	STRAND PRIMER TYPE
D2S2161	1	tgttcacccctcaggc	Forward
	2	attactcctattgtccctgtctgc	Reverse
D2S2232	3	gacggcctgtttatggtatatct	Forward
	4	agcttagattcagactgactgg	Reverse
D2S2181	5	cctcccaaaccacctg	Forward
	6	ccccattaacaaattagtcg	Reverse
D2S113	7	gcttgtttcatctcaccctg	Forward
	8	ctgttgtttttttaggtgggag	Reverse
D2S 2209	9	tcaccaatcccatcctt	Forward
	10	catgcgtatgcgtntgc	Reverse
D2S2264	11	catctcaaagggcatgtc	Forward
	12	tcgaatgaacagtgcctc	Reverse
D2S2356	13	cattacattcctacttttgccc	Forward
	14	agctgcctattcaaataccc	Reverse
D2S135	15	tgtgaagtgtggatgacgct	Forward
	16	aaaaatcaaacctccgagcc	Reverse
D2S2229	17	aactgtgattgagccacca	Forward
	18	ccccagcattttgatttaga	Reverse

MARKER NAME	SEQ. ID. NO.	STRAND PRIMER	STRAND PRIMER TYPE
CHROMOSOME 6			
D6S1600	19	agcttgtgcatgtgtgca	Forward
	20	caaagtcccagcaggttc	Reverse
D6S967	21	ccagccttggcgacagagt	Forward
	22	ggaactgtgaatctatttac	Reverse
D6S344	23	ctccagcctgggtcacta	Forward
	24	ctaatgcatgacaataatatttcca	Reverse
D6S1713	25	aatcactgttacccatagggttatc	Forward
	26	aggccaagacctctgtgc	Reverse
D6S1617	27	tgcaaaacaggcacacatac	Forward
	28	ttaatcaattttctgcaaagataaa	Reverse
D6S1574	29	aagaacttcccaaaccaat	Forward
	30	aaccatccaggacatcaa	Reverse
D6S1591	31	tgtttcagcagcataggg	Forward
	32	agagcctgtttggtgtcatc	Reverse
D6S1677	33	gtttccaagggctggg	Forward
	34	gaaatcaaaataacacatcctctg	Reverse
D6S1685	35	tacactaatggctctcctgg	Forward
	36	gccagatttctctgctgtag	Reverse
D6S1668	37	gtatagccaactgcttccaa	Forward
	38	gggtnccatttattgagatt	Reverse

EXAMPLE 3

Haplotype analyses of chromosome 2 for the *GLC1B*

The genetic map of chromosome 2 comprising the region associated with *GLC1B* is shown in Fig. 1.

Phenotypic status and segregation analyses of the *GLC1B* disease haplotype in autosomal dominant familial open-angle glaucoma pedigree PR. The pedigree with the associated haplotype is shown in Fig.2 . All living individuals were investigated for glaucoma and genotyped with microsatellite markers spanning the *GLC1B* locus. Selected AFM (Généthon) markers with their corresponding GDB number are represented and the sizes of the alleles associated with the *GLC1B* disease haplotype observed for each marker in pedigree PR are boxed in black. Glaucoma patients are depicted by solid black symbols, unaffected individuals by open symbols, and deceased subjects reported as blind by at least two independent family members by a black quadrant in the upper left corner of their respective symbols. Persons with intraocular hypertension (OHT) are represented by open symbols containing a central solid dot. A solid black box indicates the common *GLC1B* disease haplotype. The right side of each phased haplotype indicates the haplotype inherited from the father; the left side indicates the haplotype inherited from the mother.

EXAMPLE 4

Haplotype analyses of chromosome 6 for the locus *GLC6p25*

The genetic map of chromosome 6 comprising the region associated with the *GLC6p25* locus is shown in Fig. 3. Phenotypic status and segregation analyses of the *GLC6p25* disease haplotype in one specific branch of autosomal dominant familial open-angle glaucoma pedigree BV is shown in Fig. 4. All living individuals were investigated for glaucoma and genotyped with microsatellite markers spanning the *GLC6p25*locus. Selected AFM (Généthon) and UTAH (D6S967) markers with their corresponding GDB number are represented and the sizes of the alleles associated with the *GLC6p25* disease haplotype

observed for each marker in pedigree BV are boxed in black. Open-angle glaucoma patients are depicted by solid black symbols, unaffected individuals by open symbols, and OHT patients by a black quadrant in the lower left corner of their respective symbol. A black dot at lower left of a symbol represents a person participating in the study. A black dot at lower right of a symbol represents a person with abnormal tissue in the iridocorneal angle or iris hypolplasia. The solid black box indicates the common *GLC6p25* disease haplotype. The right side of each phased haplotype indicates the haplotype inherited from the father; the left side indicates the haplotype inherited from the mother. Present ages of normal and OHT patients as well as ages of affected carriers at time of diagnosis are depicted above their respective symbols.

Although the present invention has been described, in some detail by way of illustration and examples for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto, without departing from the spirit and nature of the subject invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. A method for detecting the presence in an individual of an allele for glaucoma comprising haplotype analysis of human chromosome 2 wherein said haplotype is associated with the locus *GLC1B*.

2. The method for detecting the presence in an individual of an allele for glaucoma of claim 1, wherein a chromosomal region of said chromosome 2 for haplotype analysis encompasses 11.4cM and comprises a region on the long arm of chromosome 2 between cen and q13.

3. The method for detecting the presence in an individual of an allele for glaucoma of claim 2, wherein said haplotype comprises at least two markers within said chromosome 2 region.

4. The method of claim 3, wherein said at least two chromosomal markers comprise markers designated as D2S2161 and D2S2229 from Généthon.

5. The method for detecting the presence in an individual of an allele for glaucoma of claim 4, wherein said chromosomal region as defined by said marker D2S2161 is flanked by sequences being comprised in SEQ. ID. NO.: 1 and in a sequence complementary to SEQ. ID. NO.: 2, being said amplification primers amplifying the said region of marker D2S2161, and wherein the chromosomal region as defined by said marker D2S2229 is flanked by sequences being comprised in SEQ. ID. NO.: 17 and in a sequence complementary to SEQ. ID. NO.: 18, being said amplification primers amplifying

the said region of marker D2S2229, whereby the length of the amplified nucleotide region is associated with the mutant allele.

6. The method for detecting the presence in an individual of an allele for glaucoma of claim 3, wherein the haplotype comprises nine markers within the aforementioned chromosome 2 region.

7. The method for detecting the presence in an individual of an allele for glaucoma of claim 6, wherein the haplotype comprises nine markers within the said chromosome 2 region, said nine markers are flanked by sequences being comprised in or complementary to one of SEQ. ID. NO.: 1 to SEQ. ID. NO.: 18, being amplification primers amplifying said region of markers D2S2161, D2S2232, D2S2181, D2S113, D2S2209, D2S2264, D2S2356, D2S135, D2S2229 with amplification primer pairs of SEQ. ID. NO.: 1/2, 3/4, 5/6, 7/8, 9/10, 11/12, 13/14, 15/16, 17/18, respectively.

8. The method for detecting the presence in an individual of an allele for glaucoma of claim 3, wherein the haplotype comprises nine markers within said chromosome 2 region and said locus *GLC1B* is associated with said haplotype.

9. The method of claim 8, wherein said nine markers define an inherited haplotype that is associated with said *GLC1B* locus such that a haplotype profile is associated with said mutant *GLC1B* and is a means for detecting presymptomatic or symptomatic glaucoma in said individual.

10. The method of claim 8, wherein said nine markers define an inherited haplotype that is associated with said *GLC1B* locus such that

different haplotype profile are used to localise, isolate and identify said *GLC1B* locus whereby such localisation enhances detection of individuals with glaucoma.

11. A diagnostic kit comprising the amplification primers of SEQ. ID. NO.: 1 to SEQ. ID. NO.: 18, for the haplotype analysis of individuals wherein said haplotype analyses is used for detection and prognosis of individuals with said mutant *GLC1B* locus.

12. A method for detecting the presence in an individual of an allele for glaucoma comprising haplotype analyses of human chromosome 6, wherein said haplotype is associated with the locus *GLC6p25*.

13. The method for detecting the presence in an individual of an allele for glaucoma of claim 12, wherein a chromosomal region for haplotype analysis encompasses 5cM and comprises a region on the short arm of chromosome 6 between p25 and tel.

14. The method for detecting the presence in an individual of an allele for glaucoma of claim 13, wherein said haplotype comprises at least two markers within said chromosome region.

15. The method of claim 14, wherein said at least two chromosomal markers comprise markers being designated D6S1600 and D6S296 from Généthon.

16. The method for detecting the presence in an individual of an allele for glaucoma of claim 15, wherein said chromosomal region as

defined by said marker DS1600 is flanked by sequences being comprised in SEQ. ID. NO.: 19 and in a sequence complementary to SEQ. ID. NO.: 20, being said amplification primers amplifying said region of marker DS1600, and chromosomal region as defined by said marker D6S1668 is flanked by sequences being comprised in SEQ. ID. NO.: 37 and in a sequence complementary to SEQ. ID. NO.: 38, being said amplification primers amplifying said region of marker DS1668, whereby the length of the amplified nucleotide region is associated with the mutant allele of *GLC6p25*.

17. The method for detecting the presence in an individual of an allele for glaucoma of claim 14, wherein said haplotype comprises ten markers within said chromosome region.

18. The method for detecting the presence in an individual of an allele for glaucoma of claim 17, wherein said haplotype comprises ten markers within said chromosome region, said ten markers are flanked by sequences being comprised in or complementary to one of SEQ. ID. NO.: 19 to SEQ. ID. NO.: 38, being amplification primers amplifying said region of markers D6S1600, D6S967, D6S344, D6S1713, D6S1617, D6S1574, D6S1591, D6S1677, D6S1685, D6S1668, with amplification primer pairs of SEQ. ID. NO.: 19/20, 21/22, 23/24, 25/26, 27/28, 29/30, 31/32, 33/34, 35/36, 37/38, respectively.

19. The method for detecting the presence in an individual for an allele for glaucoma of claim 14, wherein said haplotype comprises ten markers within said chromosome region and the locus *GLC6p25* is associated with said haplotype.

20. The method of claim 19, wherein said ten markers define an inherited haplotype that is associated with the said *GLC6p25* locus such that a haplotype profile is associated with a mutant *GLC6p25* and is a means for detecting presymptomatic or symptomatic glaucoma.

21. The method of claim 20 wherein said ten markers define an inherited haplotype that is associated with said *GLC6p25* locus such that different haplotype profiles are used to localise, isolate and identify the *GLC6p25* locus whereby such localisation enhances detection of individuals with glaucoma.

22. A diagnostic kit comprising the amplification primers of SEQ. ID. NO.: 19 to SEQ. ID. NO.: 38, for the haplotype analyses of individuals wherein said haplotype analyses is used for the detection and prognosis of individuals with a mutant *GLC6p25* locus.

1/4

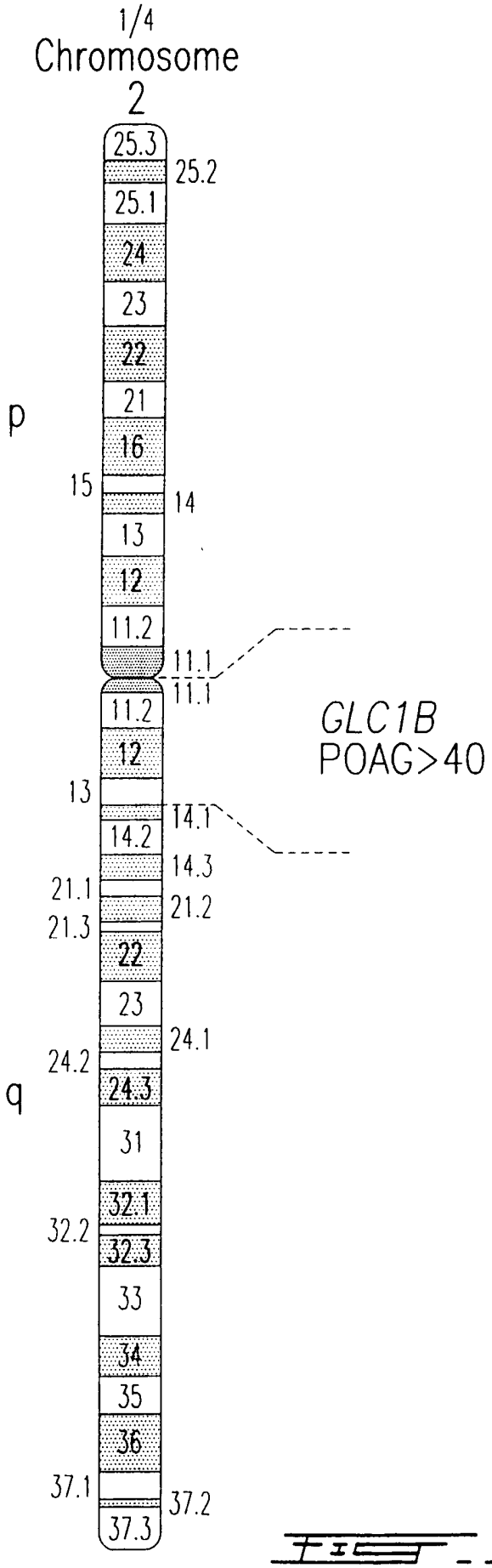

Fig. 1

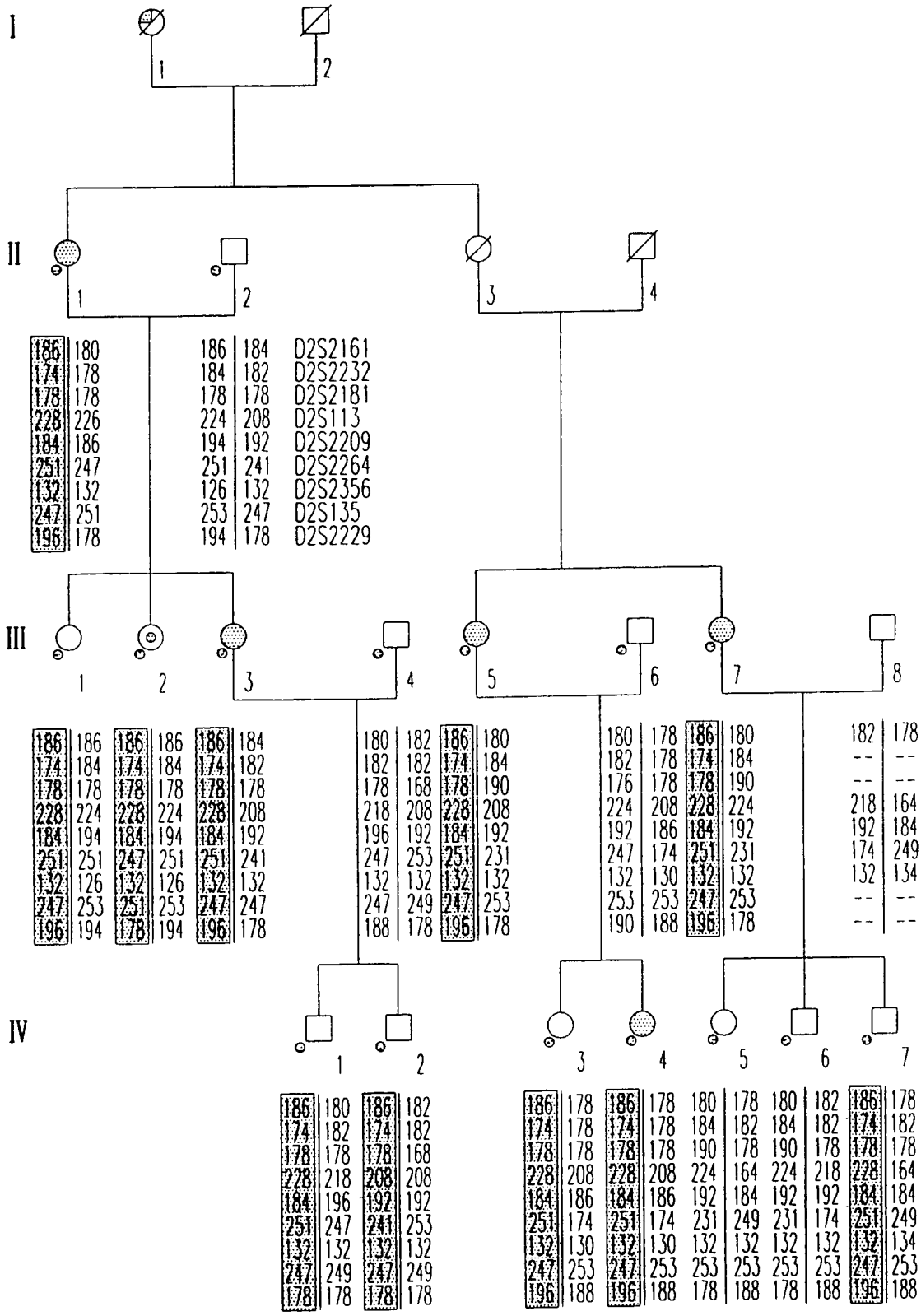

FIG. 2

6p25 GLAUCOMA LOCUS

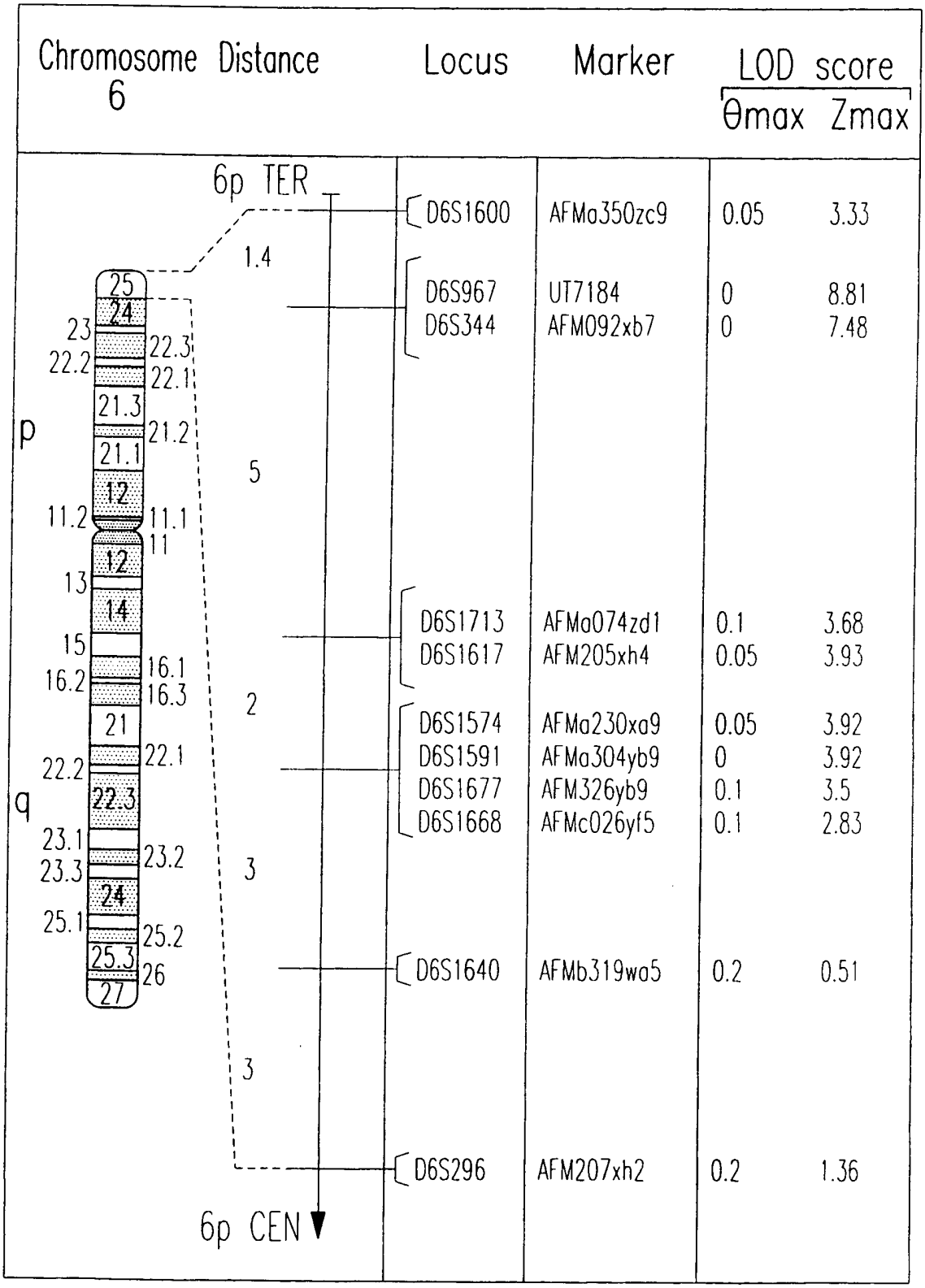

Fig. 3

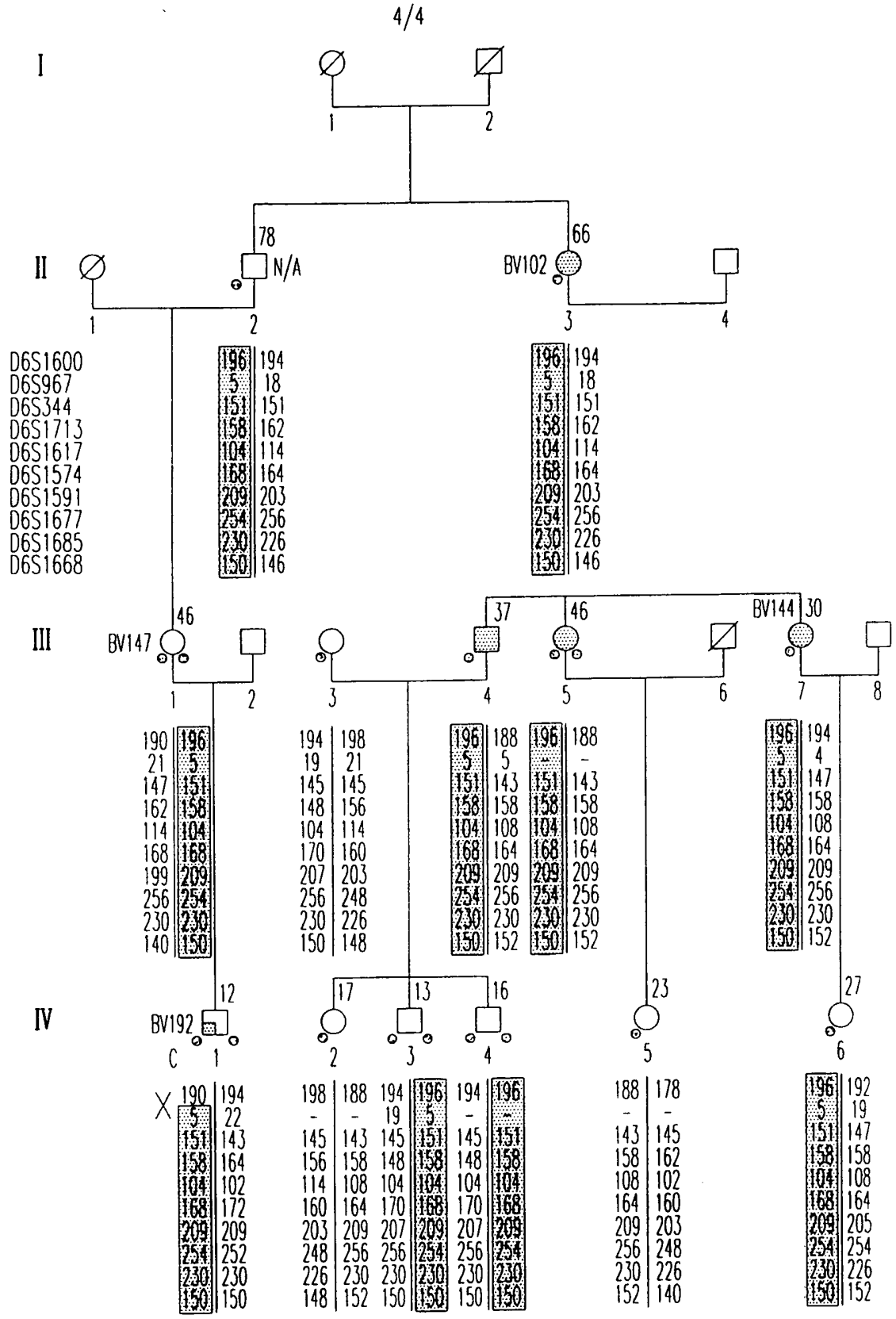

Fig. 4

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(71) Applicant *(for all designated States except US)*: UNIVERSITE LAVAL [CA/CA]; Cité Universitaire, Québec, Québec G1K 7P4 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: RAYMOND, Vincent [CA/CA]; Appartement 3, 858 Madeleine-de-Verchères, Québec, Québec G1S 4K5 (CA). MORISSETTE, Jean [CA/CA]; 3835 Jean-F. Grenon, Sainte-Foy, Québec G1S 3Y2 (CA). FALARDEAU, Pierre [CA/CA]; 1085 Marguerite-Bourgeoys, Sillery, Québec G1S 3Y2 (CA). COTE, Gilles [CA/CA]; 1395 De Godefroy, Sillery, Québec G1T 2E3 (CA). ANCTIL, Jean-Louis [CA/CA]; 1065 Marguerite-Bourgeoys, Sillery, Québec G1T 3Y1 (CA).

(74) Agents: DUBUC, Jean, H. et al.; Goudreau Gage Dubuc & Martineau Walker, The Stock Exchange Tower, Suite 3400, P.O. Box 242, Montreal, Québec H4Z 1E9 (CA).

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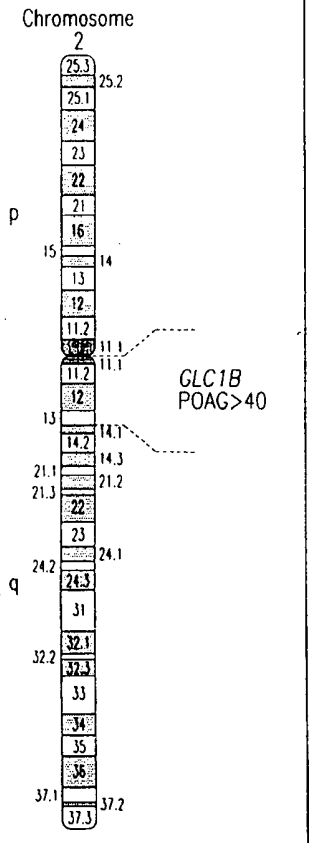
(57) Abstract

The present invention relates to haplotype analyses for the indirect detection of glaucoma. Based on the knowledge of linkage, two candidate genes for glaucoma one on chromosome 2, the other on chromosome 6, appear to be linked to a set of markers. The present invention provides the location of these markers and the primers for their amplification.



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INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/CA 98/00924

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STOILOVA D ET AL: "LOCALIZATION OF A LOCUS (GLC1B) FOR ADULT-ONSET PRIMARY OPEN ANGLE GLAUCOMA TO THE 2CEN-Q13 REGION" GENOMICS, vol. 36, no. 434, 1996, pages 142-150, XP002068985 cited in the application see the whole document --- -/--	1-11

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
8 March 1999	29/03/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Molina Galan, E

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INTERNATIONAL SEARCH REPORT

Int .tional Application No
PCT/CA 98/00924

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	STOILOVA, DILIANA (1) ET AL: "The first locus for adult-onset primary open angle glaucoma (GLC1B--) maps to the 2cen-q13 region." INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE, (1997) VOL. 38, NO. 4 PART 1-2, PP. S931. MEETING INFO.: ANNUAL MEETING OF THE ASSOCIATION FOR RESEARCH IN VISION AND OPHTHALMOLOGY, PARTS 1-2 FORT LAUDERDALE, FLORIDA, USA MAY 11-16, 199 ISSN: 0146-0404., XP002095711 see the whole document	1-11
X	MEARS ET AL.: "Autosomal dominant iridogoniodysgenesis anomaly maps to 6p25" AM J HUM GENET, vol. 59, 1996, pages 1321-1327, XP002095712 cited in the application see the whole document	12-22
X	GOULD ET AL.: "Autosomal dominant Axenfeld-Rieger anomaly maps to 6p25" AM J HUM GENET, vol. 61, September 1997, pages 765-768, XP002095713 see the whole document	12-22
A	RAYMOND, VINCENT: "Molecular genetics of the glaucomas: mapping of the first five "GLC" loci" AM. J. HUM. GENET. (1997), 60(2), 272-277 CODEN: AJHGAG;ISSN: 0002-9297, XP002095714	
A	DIB ET AL.: "A comprehensive genetic map of the human genome based on 5264 microsatellites" NATURE, vol. 380, 1996, pages 152-154, XP002095715 cited in the application	
P,X	JORDAN T ET AL: "Familial glaucoma iridogoniodysplasia maps to a 6p25 region implicated in primary congenital glaucoma and iridogoniodysgenesis anomaly." AMERICAN JOURNAL OF HUMAN GENETICS, (1997 OCT) 61 (4) 882-8. JOURNAL CODE: 3IM. ISSN: 0002-9297., XP002095716 United States see the whole document	12-22

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INTERNATIONAL SEARCH REPORT

Int .tional Application No
PCT/CA 98/00924

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	NISHIMURA, DARRYL Y. ET AL: "The forkhead transcription factor gene FKHL7 is responsible for glaucoma phenotypes which map to 6p25." NATURE GENETICS, (JUNE, 1998) VOL. 19, NO. 2, PP. 140-147. ISSN: 1061-4036., XP002095717 see the whole document ---	12-22
T	MEARS A J ET AL: "Mutations of the forkhead/winged-helix gene, FKHL7, in patients with Axenfeld-Rieger anomaly." AMERICAN JOURNAL OF HUMAN GENETICS, (1998 NOV) 63 (5) 1316-28. JOURNAL CODE: 3IM. ISSN: 0002-9297., XP002095718 United States see the whole document -----	12-22

1

INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/CA 98/00924

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

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This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-11

   Methods and kits for detecting the presence of an allele for glaucoma comprysing haplotype analysis of human chromosome 2 wherein said haplotype is associated with the locus GLC1B.

2. Claims: 12-22

   Methods and kits for detecting the presence of an allele for glaucoma comprysing haplotype analysis of human chromosome 6 wherein said haplotype is associated with the locus GL6p25.